suffering from a disease, disorder or condition of the central nervous system and wherein said human donor is [synergeneic] syngeneic with said patient.

8. (Amended) The method of claim 1, wherein prior to administering said isolated stromal cells, said cells are cultured in vitro to accomplish at least one of expansion in the number of said cells and to convert said cells into replicating cells.

REMARKS

The present invention relates to novel isolated stromal cells for use in treatment of a central nervous system ("CNS") disease, disorder or condition. The invention discloses methods comprising administering isolated stromal cells into the CNS of a human patient thereby effecting treatment of a disease, disorder or condition thereof. Moreover, the stromal cells may be cultured *in vitro*, genetically engineered to produce therapeutic compounds, and pre-differentiated prior to administration into the CNS.

Original claims 1-20, are pending in the application.

Claims 2 and 8, have been amended to more particularly point out and distinctly claim the subject matter which Applicant regards as his invention. Support for these amendments is found in the specification as filed as more fully set forth below. Thus, no new matter has been added by way of these amendments.

Information Disclosure Statement

The Examiner acknowledges entry of the Information Disclosure Statement filed on September 10, 1998. However, this was a supplemental Information Disclosure Statement and it appears that the initial Information Disclosure Statement filed on May 15, 1998, has not been made of record in this application.

Therefore, Applicants have enclosed herewith a copy of the Information Disclosure Statement filed on May 15, 1998, including a copy of the stamped post card evincing that the Information Disclosure Statement was received by the Patent Office

in May of 1998. Applicants respectfully request that the Examiner consider and make of record herein the Information Disclosure Statement originally filed on May 15, 1998, and that the Examiner initial and return a copy of the Form PTO-1449 which accompanies this document to indicate that the citations have been considered.

Rejection of Claims 1-18, Under 35 U.S.C. § 112, first paragraph

Claims 1-18 stand rejected under 35 U.S.C. § 112, first paragraph, because in the Examiner's opinion, cell and gene therapy using marrow stromal cells ("MSCs") are not enabled by the disclosure in the specification given the unpredictability of the art at the time of filing. The Examiner cites the following references in support of her rejection: Prockop (1997, Science 276:71-74), Gerson (1999, Nature Med. 5:262-264), Sanberg and Willing (1998, Nuc. Acids Symp. Ser. 38:139-142) (hereinafter referred to as "Sanberg"), and Sabaté et al. (1996, Clin. Neurosci. 3:317-321) (hereinafter referred to as "Sabate").

Applicants respectfully submit that the claimed cell and gene therapy methods are enabled by the specification as filed under the current law pursuant to 35 U.S.C. § 112, first paragraph. It is well-settled that an Applicant need not have actually reduced the invention to practice prior to filing. MPEP §2164.02 (citing *Gould v. Quigg*, 822 F.2d 1074 (Fed. Cir. 1987)). Indeed, the invention need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908 (C.C.P.A. 1970). The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. MPEP §2164.01 (citing *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976)). The fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. *Id.* Further, the specification need not disclose what is well-known to those skilled in the

art and preferably omits that which is well-known to those skilled and already available to the public. MPEP §2164.05(a) (citing *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991)). Therefore, under current law, enablement does not require a working example and experimentation is allowed so long as it is not undue.

The disclosure in the specification as filed amply supports methods of treating a human patient having a disease, disorder or condition of the CNS comprising administering isolated stromal cells to the CNS of the patient and further supports such methods comprising cells comprising an isolated nucleic acid encoding a therapeutic protein. The specification supports such methods because even though no working example is required under current law, there has been extensive reduction to practice in this instance. Further, the instant application only omits that which is well-known to those skilled in the art and that which is already known to the public.

With regard to use of MSCs in CNS cell therapy, the specification discloses that Applicants have transplanted human stromal cells into mice and detected engraftment in the marrow, spleen, bone, lung, and cartilage of the recipient animal (specification at page 35). Further, marrow stromal cells from normal mice were transplanted into osteogenesis imperfecta ("OI") mice that expressed high levels of a mutated procollagen gene (COL1 A1). One month after infusion, the normal donor cells had given rise to 10-45% of the bone cells in recipient mice. Further, the data disclosed that the bone of recipient mice demonstrated an increase in the ration of normal proα1 (I) chains to mutated proα1 (I) chains demonstrating that the nucleic acid encoding the normal therapeutic protein was expressed. Indeed, the normal therapeutic protein replaced the defective mutant protein in the bone of recipient OI mice. Thus, the disclosure in the specification demonstrates that MSCs can be successfully used for cell therapy.

Additionally, Applicants have reduced to practice the implantation of MSCs into the brains of recipient animals. That is, Applicants successfully transplanted both human and rat MSCs into the brains of rats (specification at pages

49-53). The data disclosed in the specification demonstrate that the donor cells were readily engrafted into the brain of recipient animals where the cells migrated into multiple areas including, but not limited to, the contralateral cortex, temporal lobe regions, cerebral cortex, rostrocaudal axis in the striatum, and the corpus callosum, where they remained localized. Thus, Applicants have demonstrated, for the first time, that rat and human MSCs can engraft, migrate, and survive in the brains of recipient animals.

Following the teachings of the instant invention as disclosed in the specification, a person of ordinary skill in the art would be able to engraft MSCs producing a therapeutic protein into the CNS of a human recipient thereby treating a disease, disorder or condition of the CNS in the recipient without undue experimentation. That is, the specification discloses that MSCs can engraft various tissues in a recipient including the brain where they express a therapeutic protein. Based on these teachings and methods well-known in the art, one of ordinary skill would be able to practice the claimed invention and nothing more is required under 35 U.S.C. §112, first paragraph.

Despite Applicants' substantial reduction to practice, the Examiner has raised a number of issues that she contends support a finding of lack of enablement. Preliminarily, Applicants respectfully submit that the specification need not disclose "any specific disease, disorder, or condition of the central nervous system which has been subjected to the claim-designated treatment regimen" since, as previously pointed out, no reduction to practice is required for enablement under Section 112, first paragraph (Office Action at page 3) (emphasis added). Further, with respect to the specific number of cells and route of administration, these are matters that the skilled artisan can determine without undue experimentation using methods known in the art and by experimentation typically engaged in by the art. Moreover, various routes of administration (*e.g.*, intraperitoneal, intravenous, and intracranial) are disclosed in the specification and all of them resulted in successful engraftment. Indeed, with respect

to introduction of donor MSCs into the CNS, a method of delivery of cells into the brain resulting in engraftment has been reduced to practice and is disclosed in the specification (at pages 47-49). Thus, there is extensive support for methods of introducing MSCs into the CNS in the specification as filed.

Applicants respectfully point out that, contrary to the Examiner's urging, methods of immunologically isolating the MSCs are disclosed in the specification, *e.g.*, the use of diffusion chambers to circumvent immune responses is disclosed in the specification at page 45. Further, in the case of MSCs obtained from syngeneic donors or from the patient, there are little, if any, immunologic concerns and immunological isolation is not required. Therefore, because methods of immunologically isolating MSCs are well-known in the art and are disclosed in the specification, Applicants do not understand the Examiner's contention that they are not disclosed. Further, with respect to neurotransplantation using cells obtained from the recipient or from a syngeneic donor, the immune system does not pose any impediment to use of MSCs for CNS neurotransplantation to treat a disease, disorder or condition.

Despite Applicants' extensive reduction to practice, the Examiner cites three review articles (Prockop, Gerson and Sanberg) to support that there is no enablement because, according to these references, the art of MSC transplantation is unpredictable. Applicants respectfully submit that these references do not support a finding of lack of enablement in the instant application.

Initially, Applicants respectfully point out that Prockop is not a prior art reference for purposes of 35 U.S.C. § 112, first paragraph, to demonstrate the unpredictability of the art at the time of filing. This is because Prockop, on which the co-inventor of the present invention, Darwin J. Prockop, is sole author, was published less than one year before the filing date of this application, *i.e.*, February 24, 1998. Furthermore, Applicants point out that this application is a continuation-in-part of PCT Application No. US/PCT96/04407, filed on March 28, 1996, which is a continuation of U.S. Application No. 08/412,066, filed on March 28, 1995, and which is also entitled

to priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/006,627, filed on November 13, 1995. Irrespective of any claims to priority under 35 U.S.C. §119, the Prockop reference cannot be prior art since it was published within one year of the filing date of this application.

Gerson is a review article commenting on the successful treatment of OI in children using MSCs expressing the normal proα1 gene much like the successful expression of normal proα1 in the transgenic model of OI in mice disclosed in the specification at page 37. Rather than supporting a finding of lack of enablement, this reference further supports that MSCs can be used successfully to treat human disease by gene therapy, cell therapy, or both. More specifically, the reference teaches that Horwitz et al. (1999, Nature Med. 5:309-313), a reference co-authored by Darwin J. Prockop who is a co-inventor in the present application, demonstrates that whole bone marrow, which contains MSCs, can be used to replace a defective gene product with the normal wild type product resulting in clinical improvement of a disease phenotype (*e.g.*, increased mineral content of bones, evidence of bone growth, and decreased rates of fractures in children with severe OI). Thus, Gerson supports that MSCs can be used for cell therapy to successfully treat a disease, disorder or condition in a human patient and that the art typically engages in such experimentation.

The Examiner contends that Gerson poses a number of questions which indicate that the art of MSC cell and gene therapy is unpredictable. Although Gerson does note some issues concerning use of MSC in cell and gene therapy at page 264, left column, it is not clear that these questions must be answered to satisfy the enablement requirement of 35 U.S.C. §112, first paragraph. More importantly, Gerson documents that the art regularly engages in such experimentation to determine the answers to the questions posed. Indeed, the data disclosed in the instant specification address the questions of to which tissues do infused MSCs home, proliferate and differentiate, and which regulatory signals are used. That is, the data disclosed demonstrate that donor MSCs implanted in the recipient brain were found in multiple areas of the brain and

behaved similarly to implanted astrocytes. Further, the data disclosed demonstrate that the MSCs ceased synthesis of type I collagen after integration into brain tissue. Therefore, even assuming the questions posed by Gerson are relevant to enablement under current patent law, several of these questions are addressed by the data disclosed in the specification as filed.

Sanberg is another review article discussing various therapeutic approaches to neurodegenerative disorders. The Examiner contends that Sanberg supports a finding of lack of enablement since the article notes that host immune response to the grafted tissue presents a serious problem. However, this is one of the advantages of the present invention in that MSC can be readily obtained from a syngeneic donor or from the patient being treated such that host immune response to the transplanted MSCs is circumvented. Further, immunological isolation of the MSCs using, for example, diffusion chambers, also can be used to circumvent immune response to the grafted cells.

The Examiner also points out that Sanberg notes the difficulty of treating Hungtington's Disease because the disorder involves extensive cell death throughout the brain. However, the data disclosed in the specification demonstrate that MSCs introduced into the brain migrate and localize throughout the brain making MSCs ideal for treatment of diseases involving various areas of the brain. Moreover, MSCs can be readily expanded in culture without loss of multipotential making these cells ideal for situations where large numbers of cells must be administered. Thus, rather than support that treatment of CNS disease using MSC-based cell therapy is not enabled, Sanberg, when read in light of the disclosure provided in the specification, actually supports that MSCs can be used for such treatments and, indeed, use of MSCs overcomes the deficiencies of the prior art.

In addition, the Examiner has taken several sentences out of context from Sanberg giving the impression that cell therapy for CNS disease is but an unrealized dream. However, Sanberg, at the last paragraph on page 141, concludes with the following four sentences:

> **Conclusions:** From the data presented, it should be clear that cell transplantation has a place in our arsenal of therapeutic treatments for neural degenerative diseases and stroke. The future of the field probably rests with the transplantation of stem cells or cells derived from immortalized cell lines. Techniques to enhance neuronal survival through delivery of trophic or immunosuppressive factors will also continue to be important. The potential for this line of research to eventually be used in the clinic as therapeutic treatments for degenerative diseases is becoming even more likely as development is facilitated by industrial funding.

Therefore, far from supporting that the art of cell therapy using MSCs is unpredictable, Sanberg acknowledges that cell therapy for treatment of CNS disease involving stem cells is presently an alternative in our present arsenal of therapeutics. Thus, Sanberg does not support the rejection of the claims based on 35 U.S.C. §112, first paragraph.

With regard to enablement of *ex vivo* gene therapy using MSCs for neurotransplantation, the Examiner cites a review article by Sabate et al. to support the rejection of the claims based upon lack of enablement. As stated previously elsewhere herein, Applicants have demonstrated that MSCs can be cultured and expanded *in vitro* while retaining their stem-cell like phenotype (specification at page 36), and can be stably transfected with exogenous nucleic acids using standard methods (*e.g.*, retrovirus infection, lipofectamine transfection, and the like) (specification at pages 37-38, 40-41). Moreover, as stated previously elsewhere herein, the cells have been used to successfully engraft various tissues of recipient animals (specification at pages 33-35, and 37) including the brain (specification at pages 47-53).

The portion of Sabate cited by the Examiner as pointing out the pitfalls of using adenoviral vectors to treat CNS disease is inapposite to the instant application. That is, while viral transduction is a technique which can be used to introduce an isolated nucleic acid encoding a therapeutic protein into MSCs which are then implanted in a human patient, Sabate deals with introducing adenoviral vectors directly into the CNS of a patient to introduce a nucleic acid of interest into the cells of the

CNS of the patient. This approach, as noted by Sabate at page 318, left column at bottom, has obvious disadvantages and problems which must be overcome. However, most of these concerns (*e.g.*, targeting of the cells, safety of the procedure, vector large-scale production capacity) are not germane to the use of recombinant MSCs that express a transgene since cell therapy does not involve use of a virus pathogen. Indeed, Sabate discusses the advantages of using an *ex vivo* gene therapy approach but suggests using human neural progenitors obtained from aborted fetuses instead of MSCs. As pointed out previously, using MSCs obviates any ethical and technical hurdles involved in using fetal-derived cells. Thus, Sabate, which addresses the problems associated with using adenoviral vectors for direct gene therapy, is not relevant to the instant invention which uses MSCs comprising an isolated nucleic acid where the nucleic acid can be introduced into the cells by a variety of methods many of which do not involve use of viral vectors.

Additionally, the Examiner contends that the claims are not enabled because the specification does not provide guidance as to which nucleic acid sequences are suitable for encoding any and all therapeutic proteins, and does not provide information as to a correlation between a mutated, non-functional, or underexpressed gene and a CNS disease, disorder or condition. However, Sabate at page 317, provides an extensive list of gene products where the mutation, non-function or underexpression thereof mediates a CNS disease, disorder or condition. Sabate further notes that although "the discovery of major mechanisms implicated in neurodegenerative has opened the way to the development of new therapeutic strategies" the constraints posed by the blood-brain barrier in delivery of therapeutic molecules to the CNS has hampered used of this discovery for treatment of CNS disease (Sabate at pages 317-318). This is precisely the hurdle that the present invention overcomes in that MSCs expressing a gene of interest can be introduced directly into the CNS where the cells migrate and integrate. Thus, the gene products which mediate various disease conditions of the CNS are known in the art and such teachings of that which is well-

known in the art has been properly omitted from the specification. Therefore, Applicants need not enumerate every nucleic acid sequence and every CNS disease, disorder or condition mediated by the nucleic acid since such knowledge in known by those skilled in the art. Once armed with the teachings of the present invention, a person of ordinary skill in the art would be able to apply the methods disclosed in the instant application to treat a disease, disorder or condition of the CNS without undue experimentation.

Similarly, the selection of a specific promoter/regulatory region to be used to express an isolated nucleic acid encoding a therapeutic protein is well-known in the art and need not be taught in the instant application. Thus, this need not be disclosed in the specification.

Applicants respectfully submit that in light of the disclosure provided, the extensive reduction to practice disclosed in the specification as filed, and the state of the art of cell therapy using MSCs, claims 1-18 are enabled under 35 U.S.C. §112, first paragraph.

Even assuming, *arguendo*, that the claims are not enabled by the specification as filed, Applicants have subsequently further reduced their invention to practice. Applicants have attached a copy of a reference (Schwarz et al., 1999, Hum. Gene Ther. 10:2539-2549) co-authored by Darwin J. Prockop and S. Ausim Azizi, who are co-inventors of the present invention, disclosing additional data in support of the fact that claims 1-18 are enabled.

More specifically, Schwarz et al. demonstrate that rat and human MSCs transduced with retroviruses encoding two therapeutic proteins (*i.e.*, tyrosine hydroxylase and GTP cyclohydrolase I) express the proteins. Further, Schwarz et al. demonstrate that the transduced cells synthesized 3,4-dihydroxyphenylalanine (L-DOPA) both *in vitro* and *in vivo* upon injection of the cells into the striatum of 6-hydroxydopamine-lesioned rats which is an art-recognized rat model of Parkinson's disease. Additionally, there was a significant reduction in apomorphine-induced

rotation compared with control rats. Moreover, the data disclosed demonstrate that the cells survived for at least 87 days in the brains of recipient rats. The therapeutic proteins were expressed in the recipient brain for about 9 days, which is consistent with reports from other researchers that the retrovirus vector used mediates this transient expression. Thus, selection of a different vector should allow long-term expression of the therapeutic proteins. In sum, the data disclosed in Schwarz et al. demonstrate the successful use of MSCs comprising an isolated nucleic acid encoding a therapeutic protein in treating a CNS disease, disorder or condition (*e.g.*, Parkinsonism). Schwarz et al. is representative of the level of skill in the art and demonstrates that the skilled artisan, armed with the teachings of the instant invention, would be able to practice the invention commensurate with the scope of the claims without undue experimentation.

Thus, based upon the disclosure provided in the specification and methods well-known in the art, one of ordinary skill would be able to introduce an isolated nucleic acid encoding a therapeutic protein into MSCs and then engraft the CNS of a human recipient using the recombinant MSCs thereby treating a disease, disorder, or condition of the CNS mediated by the therapeutic protein without undue experimentation. Therefore, claims 1-18 are enabled under 35 U.S.C. §112, first paragraph, and the rejection of these claims for lack of enablement should be reconsidered and withdrawn.

Rejection of Claims 1-20, Under 35 U.S.C. § 112, second paragraph

Claims 1-20 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants, on the other hand, respectfully submit that these claims are not indefinite in any way under the current law pursuant to Section 112, second paragraph.

It is settled law that the "patent law allows the inventor to be his own lexicographer." *Chicago Steel Foundry Co. v. Burnside Steel Foundry Co.*, 132 F.2d 812 (7th Cir. 1943). *See also* MPEP ə 2173.01. This is because "[t]he dictionary does

not always keep abreast of the inventor. It cannot. Things are not made for the sake of words, but words for things." *Autogiro Co. v. U.S.*, 155 USPQ 697 (Ct. Cls. 1967). Further, applicant is entitled to have the claims construed in connection with the other parts of the application. *See Autogiro Co. v. U.S.*, 155 USPQ 697 (Ct. Cls. 1967). Therefore, Applicants are entitled to define terms to describe their invention and the claims must be interpreted in light of the other parts of the application including the disclosure in the specification and the definitions provided therein.

Applicants respectfully submit that when claims 1-20 are interpreted in light of the disclosure of the specification and the definitions set forth therein, it is clear that these claim are in no way indefinite. The Examiner contends that claim 1 is vague and indefinite in that "it is unclear what CNS diseases, disorders, or conditions are suitable for treatment by administering isolated stromal cells; it is unclear how the presence of the cells effects treatment of the disease, disorder or condition; and it is unclear what clinical parameters are to be measured such that a person with a disease, disorder or condition can be identified" (Office Action at page 6).

Applicants respectfully submit that the disclosure provided in the specification makes it abundantly clear which diseases, disorders or conditions of the CNS are encompassed as well as how the presence of the cells effects treatment and what clinical parameters are to be measured and that which is not explicitly stated is well known in the art. For instance, the specification at pages 16-22 provides extensive disclosure including a list of several CNS diseases, disorders or conditions which can be treated using MSCs. Such diseases, disorders or conditions include, but are not limited to, genetic diseases of the CNS (*e.g.*, Tay-Sach's, Sandhoff's disease, Hurler's syndrome, Krabbe's disease), birth-induced traumatic CNS injury (specification at page 16, lines 8-21), as well as adult CNS diseases, disorders or conditions, *e.g.*, Parkinson's, Alzheimer's, and Huntington's diseases, epilepsy, amyotropic lateral sclerosis, multiple sclerosis, trauma, tumors, stroke, and the like (specification at page 16, lines 23-27) and including degenerative diseases and traumatic injury of the spinal cord. Thus,

when the claims are read in light of the other parts of the application, these claims are not indefinite for failure to set forth what CNS diseases, disorders, and conditions are encompassed within the scope of the claims. Applicants respectfully submit that every disease, disorder, or condition need not be enumerated to satisfy the requirements of 35 U.S.C. §112, second paragraph, where numerous examples are provided and where a person of ordinary skill in the art would know which diseases, disorders or conditions lie within the claims.

Further, claim 1 is not indefinite since it is not unclear how the isolated MSCs are administered for each CNS disease, disorder or condition. That is, as exemplified in the specification by the injection of MSCs into the brains of recipient rats, the disclosure provides for transplantation of the cells directly into the CNS (*see also* specification at page 16, lines 8-22). Further, the specification makes clear that the cells can be delivered to the site of brain tumors and spinal cord lesions (specification at page 17, lines 1-8). Additionally, the specification discloses that the cells can be introduced into the CNS by creating a hole in the cranium through which the cells can be passed, as well as by direct injection, using a shunt, or by any other means used in the art to introduce compounds into the CNS (specification at page 15, lines 8-13). Moreover, the skilled artisan, armed with the teachings of the instant invention, would not be confused as to how to determine the route of administration for each disease, disorder, or condition which route is dependent on the disease, disorder or condition being treated, so that these teachings known to one skilled in the art need not be disclosed in the specification.

Further, it is not unclear how the presence of the cells effects treatment of the disease, disorder, or condition. Indeed, there is ample disclosure in the specification discussing how the presence of MSC effects treatment whether by replacing defective cells, by supplementing, augmenting and/or replacing defective cells with cells that correctly express a normal gene, to replace or supplement the brain cells in an individual comprising a CNS tumor, to produce a therapeutic protein thus

administering the desired protein to the CNS, to express desired secreted proteins which exert a biologically active therapeutic or prophylactic effect (specification at page 16, line 8, to page 22, line 5). Thus, the specification makes it abundantly clear how the presence of the MSC effects treatment of a CNS disease, disorder or condition and there is nothing vague and indefinite about claim 1 in this regard.

In addition, the extensive disclosure concerning how the presence of MSCs effects treatment further supports that it would not be unclear to one skilled in the art, armed with the teachings of the instant invention and the knowledge of the prior art, what diseases, disorders or conditions of the CNS are suitable for treatment by administering isolated stromal cells. That is, knowing how the present invention works makes clearer to the skilled artisan which diseases, disorders, or conditions will respond to the treatment methods disclosed by Applicants in the present application. Thus, for a CNS disease, disorder, or condition for which the disease mechanism is understood, one skilled in the art, based upon the disclosure provided in the specification, would be able to determine whether the disease is amenable to such treatment.

Even assuming, *arguendo*, that it was not clear how the cells effect treatment, the present patent law does not require that the mechanism as to how the invention functions be disclosed. In *in re Cortright*, 49 USPQ2d 1464, 1469 (Fed. Cir. 1999), the Court of Appeals for the Federal Circuit discussed the enablement requirement of 35 U.S.C. §112, first paragraph and noted that

> "[I]t is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works. *Newman v. Quigg*, 877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989*); see also Fromson v. Advance Offset Plate, Inc.*, 720 F.2d 1565, 1570, 219 USPQ 1137, 1140 (Fed. Cir. 1983) ("[I]t is axiomatic that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests.").

Therefore, the Examiner cannot circumvent the Court's mandate that an applicant need not set forth, or even know, how or why the invention works under Section 112, first paragraph, by requiring the applicant to provide such information under Section 112, second paragraph. Thus, although Applicants have disclosed how the present invention works, they are not required to do so in order to patent their invention, and failure to provide such disclosure cannot form the basis for rejection under the first or second paragraph of Section 112 of the patent statute.

Thus, given the disclosure provided in the specification and well-known methods in the art, claim 1 is in no way vague and indefinite. Therefore, rejection of claim 1 under 35 U.S.C. §112, second paragraph, which merely reiterates the Examiner's rejection under 35 U.S.C. §112, first paragraph, for lack of enablement, should be reconsidered and withdrawn since the claims, when read in light of the other parts of the application, are not vague and indefinite.

The Examiner contends that claim 2 is vague and indefinite in that, in the Examiner's view, it is unclear what clinical parameters are to be measured to identify a person without a CNS disease, disorder or condition. Further, the Examiner contends that "synergeneic" is neither art-recognized nor defined in the specification.

Applicants respectfully submit that it would not be vague or indefinite to one skilled in the art to determine clinical parameters to determine a person without a CNS disease, disorder, or condition. Preliminarily, the term "disease, disorder, or condition of the central nervous system" is defined in the specification at page 9, line 16, to page 10, line 2. Based on the disclosure provided in the specification and diagnostic methods well-known in the art, it would not be vague and indefinite for one skilled in the art to determine a person without CNS disease, disorder or condition. Clinical diagnostic methods to detect abnormal brain structure (CAT scan, EEG, x-ray analysis, and such), cell pathology (*e.g.*, biopsy and micropathology), altered expression of molecules (*e.g.*, L-DOPA, beta-amyloid protein, and the like), as well as methods to assess the level of motor and cognitive function, are well-known in the art.

Such methods can be used to determine a person who is not suffering a CNS disease, disorder, or condition and to identify a patient who is afflicted. Obviously, with regard to disease, disorders or conditions associated with a brain tumor, spinal cord injury, brain trauma, and neurodegenerative diseases, disorders, or conditions, the state of the art of diagnostics is such that one skilled in the art, based upon the disclosure provided in the specification, would not find it unclear as to what clinical parameters are to be measure to identify a person without a CNS disease, disorder or condition. Thus, claim 2 is in no way vague and indefinite and rejection of this claim under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

Applicants, in a good faith effort to expedite prosecution in this application, have amended claim 2 to correct the typographical error pointed out by the Examiner such that the claim now recites "syngeneic." The term is a well-known term of art and this amendment is supported by the specification as filed (specification at page 14, line 18) so that no new matter has been added by way of this amendment.

The Examiner contends that claim 4 is also vague and indefinite in that, in the Examiner's view, it is unclear which genetic diseases, tumors or traumas can be effectively treated with administration of isolated stromal cells. The Examiner further contends that it is unclear how the genetic diseases, tumors or traumas which can be treated are identified. Applicants respectfully submit that claim 4 is not vague and indefinite in any way. The specification as filed makes clear starting at page 9 that isolated stromal cells can be used to replace CNS cells lost as a result of genetic disease, trauma, or other injury. As pointed out previously elsewhere herein with respect to enablement, the types of diseases, disorders, or conditions which are treatable using MSCs introduced directly into the CNS are set forth starting at page 16, line 8. They include genetic diseases of the CNS (*e.g.* Tay-Sachs disease, Krabbe's disease, Sandhoff's disease, and the like), birth-associated trauma, adult diseases of the CNS (*e.g.*, Parkinson's, Huntington's, Alzheimer's, ALS, epilepsy, and such), spinal cord injuries, and CNS tumors. Based upon the disclosure provided in the

specification, one skilled in the art would appreciate which diseases, disorders or conditions can be effectively treated using MSCs and how these can be identified.

Further, the specification at pages 15-22 teaches the mechanism by which MSCs effect treatment (*e.g.*, by replacing cells lost due to a disease, disorder, or condition, by producing a molecule either not produced, not produced in sufficient amounts, or not produced in functional form, by mediating arrest of tumor growth and/or apoptosis, and the like). Based upon this extensive disclosure of how MSCs effect their therapeutic effects, one skilled in the art would not find it unclear as to which genetic diseases, tumors or traumas can be effectively treated using MSCs or how such diseases, tumors or traumas can be identified. Thus, claim 4 is not vague and indefinite in any way and the rejection under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner also contends that claim 5 is vague and indefinite in that, in the Examiner's opinion, it is "unclear what types of injuries and to what cell types are suitable for treating by administration of isolated stem cells" (Office Action at page 7). Applicants respectfully point out that numerous types of injuries, *i.e.*, birth-related and other traumas, strokes, and spinal cord injuries, which can be treated using MSCs directly administered into the CNS are disclosed in the specification as filed (*see*, *e.g.*, specification at pages 15-20). As to what cells are suitable for treating by administration of isolated MSCs, the specification discloses but is not limited to cells that have been lost due to a CNS disease, disorder or condition which can be replaced using isolated MSCs (*see*, *e.g.*, specification at page 15, lines 14-27), neurologically defective cells which do not express sufficient or normal molecules (*see*, *e.g.*, specification at page 17, line 28, to page 18, line 7), defective tumor cells (*see*, *e.g.*, specification at page 17, lines 8-14), replicating tumor cells (*see*, *e.g.*, specification at page 18, lines 21-25). Thus, the disclosure provided in the specification makes it amply clear what types of injuries and what cell types are suitable for treating by administration of isolated stromal cells. Therefore, claim 5 is not vague and indefinite

and the rejection of this claim under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner contends that claim 6 is vague and indefinite in that, in the Examiner's view, it is unclear what types of brain tumors are amenable to treatment using isolated stem cells. Applicants respectfully submit that claim 6 is in no way vague and indefinite in this regard. Specifically, the specification discloses that MSCs can be administered to the CNS to treat brain tumors by replacing or supplementing brain cells in the patient (specification at page 18, lines 15-20), by delivering a gene product which mediates tumor cell death or arrest of tumor cell growth (specification at page 8, lines 21-25), or both. Based upon the disclosure provided in the specification, one skilled in the art would understand whether these effects would be effective to treat a tumor of interest. The skilled artisan would also take various factors into consideration in determining which approach to use involving MSCs including, but not limited to, the type of tumor, the age and condition of the patient, and the like. Thus, the extent of the disclosure provided in the specification and the methods well-known in the art render claim 6 neither vague nor indefinite since the skilled artisan would understand which brain tumors are amenable to treatment using isolated stem cells. Therefore, the rejection of claim 6 under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

In addition, the Examiner contends that claim 7 is vague and indefinite in that the term "remain present" is recited therein. This is because, according to the Examiner, the phrase "remain present" is unclear as to whether the cells "do not migrate from an area, do not differentiate, or are not subjected to an immune response which results in the lysis of the cells" (Office Action at page 7). Applicants respectfully submit that the disclosure in the specification makes the term "remain present" abundantly clear. At page 15, line 19, the specification makes clear that "the cells become permanent residents of the central nervous system." Indeed, the data disclosed in the specification at pages 47-53, demonstrate that donor MSCs

transplanted into the CNS can migrate and persist in various regions of the recipient brain and can give rise to progeny cells. Nonetheless, the data disclosed in the specification demonstrate that the cells can be detected using, for example, antibodies specific for a marker present on the donor cells but not on the recipient cells. Therefore, based upon the disclosure in the specification, the term "remain present" is not vague and indefinite since the skilled artisan would understand that the cells can migrate and persist in the CNS and can be detected in the CNS by standard methods. Thus, the rejection of claim 7 under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner further contends that claim 8 is vague and indefinite as it is unclear if the *in vitro* culturing step is to expand the isolated stromal cells or to differentiate the cells prior to administration. Applicants, while not agreeing with the Examiner's position, have amended claim 8 in a good faith effort to expedite prosecution in this application. Claim 8 as amended now recites that the cells are cultured *in vitro* to accomplish at least one of the following: expand the number of cells, allow the cells to convert from non-cycling to replicating cells (specification at page 27, lines 1-4), differentiate the cells into a cell type of choice prior to administration (specification at pages 53-56). Thus, the amendment is amply supported by the specification and no new matter has been added thereby. Since claim 8 as amended is not vague and indefinite, the rejection of this claim under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner argues that claims 9 and 11 are vague and indefinite since, in the Examiner's view, it is unclear what therapeutic protein is intended as it is unclear what type of disease, disorder or condition is being treated, and it is unclear if the nucleic acid is homologous or heterologous or from what source the nucleic acid is obtained, and it is further unclear how the protein is expressed and secreted. As stated previously elsewhere herein, based upon the disclosure provided in the specification, one skilled in the art would not be unclear as to what disease, disorder or condition can

be treated using direct transplantation of isolated stem cells. Indeed, a large number of CNS diseases, disorders or conditions that can be treated using MSCs is set forth in the specification as filed such that the disease, disorder or condition includes, but is not limited to, stroke, trauma, brain tumor, Alzheimer's, Parkinson's, Huntington's, Krabbe's, Tay-Sachs, and Sandhoff's diseases, Hurler's syndrome, epilepsy, birth trauma, spinal cord injury, and amyotropic lateral sclerosis.

Given that the skilled artisan would not be confused about what CNS disease, disorder or condition can be effectively treated using MSCs, claims 9 and 11 are not vague and indefinite as to what therapeutic protein is intended. Indeed, Schwarz et al., *supra*, following the teachings of the present specification, transduced isolated MSCs with a vector comprising a therapeutic protein which when expressed, mediate the production of L-DOPA in the isolated stem cells. The MSCs which have been co-transduced with both nucleic acids produced L-DOPA both *in vitro* and *in vivo*. Indeed, engraftment of the transduced MSCs expressing the therapeutic proteins mediated a detectable improvement in the condition of rats in an art-recognized model of Parkinson's disease.

Applicants do not understand the Examiner's confusion as to whether the nucleic acid is homologous or heterologous or from what source it is obtained since it would be clear to one skilled in the art, based upon the disclosure provided in the specification, that in order for the therapeutic protein to provide its therapeutic effects, it must be functional in the recipient. The source of the nucleic acid and whether the nucleic acid is heterologous or homologous to the recipient is irrelevant so long as the therapeutic protein effects beneficial effects in the recipient comparable to the effects of expression of the normal protein. Indeed, the invention encompasses treatments where higher levels of the therapeutic protein compared to the endogenous levels of the same protein are desired in order to effectively treat the disease, disorder or condition (specification at page 21, lines 8-9). One skilled in the art would understand where to obtain an isolated nucleic acid encoding a therapeutic protein of interest in order to

treat a CNS disease, disorder or condition mediated by a lack or decreased amount of the protein or by the presence of non-functional protein.

Additionally, Applicants do not understand the Examiner's concern regarding how the protein is expressed with regard to claims 9 and 11. That is, claims 9 and 11 do not recite that the protein is expressed; rather, both claims recite that the protein effects treatment of the disease, disorder or condition when the protein is expressed. Thus, claims 9 and 11 do not recite that the protein is expressed or how such expression is effected and these are claim limitations of claims depending from claims 9 and 11.

In light of the foregoing, Applicants respectfully submit that claims 9 and 11 are not vague and indefinite and, therefore, the rejection of these claims under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner contends that claims 12 and 15 are vague and indefinite since it is unclear which promoter/regulatory sequence is suitable for expression in isolated stromal cells. Applicants respectfully submit that selection of a promoter/regulatory sequence to effect expression (*e.g.*, promoter/regulatory sequences which drive constitutive or tissue-specific expression of a desired nucleic acid sequence) are well-known in the art and need not be disclosed to one of ordinary skill in the art. The specification at pages 22-24sets forth a number of promoter/regulatory sequences useful for driving the expression of a therapeutic protein in MSCs. Indeed, expression of two therapeutic proteins in MSCs such that a detectable improvement in a rat model of Parkinson's disease was achieved, has been reduced to practice by Schwarz et al., *supra*, following the teachings provided in the specification as filed. Therefore, one skilled in the art, based upon the disclosure provided in the specification and methods well-known in the art, would understand how to select an appropriate promoter/regulatory sequence to effect expression of a therapeutic protein in MSCs. Thus, claims 12 and 15 are not vague and indefinite in any way and the rejection of

these claims under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner contends that claim 15 is also vague and indefinite since it is unclear which mutated, non-functioning or under-expressed gene is intended as it is unclear how a mutated, non-functioning, or under-expressed gene is correlated to a CNS disease, disorder or condition. Applicants respectfully submit that the correlation between CNS disease, disorder, or condition and a gene product is well-known for a plethora of CNS diseases, disorders or conditions. For example, many of these genetic bases for CNS diseases, disorders or conditions are enumerated in Sabate et al., at page 317, cited by the Examiner in support of the rejection for lack of enablement. Therefore, the genetic basis for many CNS diseases, disorders, or conditions is well-known and the potential for using gene and cell therapy to treat these is well-known and recognized in the art.

Further, Applicants have subsequently further reduced the invention to practice such that two isolated nucleic acids have been introduced into MSCs and the recombinant MSCs have been successfully engrafted into a rat model of Parkinsonism thereby mediating treatment of the disease. Thus, claim 15 is not vague and indefinite since one skilled in the art, armed with the disclosure provided in the specification and the methods well-known in the art, would understand which mutated, non-functioning or under-expressed gene is intended in the effective treatment of a CNS disease, disorder, or condition. Therefore, the rejection of claim 15 under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner further contends that claim 16 is vague and indefinite since, among other things, it is unclear what is intended by "pre-differentiated," *i.e.*, it is unclear what morphologic and phenotypic markers delineate isolated stromal cells from pre-differentiated cells from differentiated cells (Office Action at page 7). Applicants respectfully point out that the term "pre-differentiated" is not vague and indefinite since it is defined clearly in the specification at page 13, lines 23-26, as

follows:

> As used herein, the term "pre-differentiated" should be construed to mean isolated stromal cells which are cocultured with a substantially homogeneous population of differentiate cells such that the isolated stromal cells differentiate and acquire phenotypic characteristics of the differentiated cells.

Therefore, the cells are pre-differentiated in that they are differentiated prior to transplantation. Further, the cells are pre-differentiated by coculturing them with a population of differentiated cells. In addition, pre-differentiation of isolated stromal cells by coculturing them with differentiated cells has been reduced to practice as disclosed in the specification at page 53, line 13, to page 56, line 14. The data disclosed in the specification exemplify what is meant by the term "pre-differentiate." Moreover, the disclosure provided in the specification demonstrates what morphologic and/or phenotypic markers distinguish isolated stromal cells from pre-differentiated cells from differentiated cells (*e.g.*, expression of glial fibrillary acidic protein, fibronectin, collagen I, HLA-ABC, vimentin, galactocerebrosidase C, and von Willenbrand Factor). However, one skilled in the art would appreciate, based upon the disclosure provided in the specification, that the specific morphologic and/or phenotypic markers will vary according to what differentiated cell type is cocultured with the isolated stromal cells and what cell type the stromal cell is being directed to differentiate into. The specific markers and assays to evaluate pre-differentiation are disclosed in the specification or are well-known to one of ordinary skill in the art.

Similarly, with regard to the Examiner's opinion that claim 16 is indefinite because it is unclear what is required in the culture steps to direct differentiation, what is intended by "substantially homogeneous" and the method of obtaining the substantially homogeneous population, and which differentiated cells are required (as well as their source), all of these teachings are set forth in the specification as filed and/or are well-known in the art. Indeed, as stated previously elsewhere

herein, the directed pre-differentiation of isolated stromal cells into cells comprising markers and phenotypic characteristics of astrocytes by coculturing the isolated stromal cells with a substantially homogeneous population of astrocytes has been reduced to practice.

Additionally, the term "substantially homogeneous population of differentiated cells" is defined in the specification at page 14, lines 3-5. The term means a population of cells "wherein at least 75% of the cells exhibit the same differentiated phenotype." Given the state of the art where cells can be readily selected and isolated based on characteristics (*e.g.*, cell sorting using surface markers, and the like), one of ordinary skill in the art would not be confused by the term "substantially homogeneous" as defined in the specification nor would the skilled artisan be confused as to how to obtain such a cell population from a source. Indeed, as pointed out previously elsewhere herein, the invention has been extensively reduced to practice including coculturing isolated stromal cells with a substantially homogeneous population of astrocytes thereby mediating pre-differentiation of MSCs into cells having at least one characteristic of an astrocyte.

Therefore, given the extensive reduction to practice in the instant application and the knowledge of the art, claim 16 is not vague and indefinite in any way and the rejection of this claim under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

The Examiner contends that claim 17 is vague and indefinite in that it is unclear what is intended by the term "pre-differentiated," *i.e.*, it is unclear, in the Examiner's view, what morphologic and phenotypic markers delineate isolated stromal cells from pre-differentiated cells from differentiated cells and it is unclear how to perform the pre-differentiation or which nucleic acid is required in the introduction step and how it is introduced. Applicants respectfully point out that the term "pre-differentiated" is defined in the specification at page 13, lines 23-26. Further, as pointed out previously, the disclosure in the specification makes clear how the pre-

differentiation is carried out and discloses assays useful for determining that the cells have differentiated as defined in the specification. Indeed, the invention has been extensively reduced to practice including the coculturing of MSCs with rat astrocytes to cause pre-differentiation of the MSCs into cells having certain characteristics of astrocytes (*see* Example 8 in the specification at pages 53-56). Therefore, claim 17 is not vague and indefinite in any way as to how pre-differentiation is to be performed and what morphologic and phenotypic markers delineate the various cells given the extensive reduction to practice and methods well-known in the art.

Further, it would not be unclear to one skilled in the art based upon the disclosure provided in the specification which nucleic acid is required in the introduction step. As stated previously elsewhere herein, nucleic acids encoding therapeutic proteins useful for treating a CNS disease, disorder or condition are well known in the art. *See, e.g.*, Sabate et al., *supra*. Further, the specification sets forth a variety of genetic and other diseases, disorders and conditions that can be treated by inserting an isolated nucleic acid into isolated stromal cells and then transplanting the transfected stromal cells into a human patient. *See, e.g.*, specification at pages 37-53. Moreover, the present invention has been further reduced to practice by Schwarz et al., *supra*, wherein following the teachings of the instant application, two nucleic acids encoding two therapeutic proteins (*i.e.*, tyrosine hydroxylase and GTP cyclohydrolase I) have been introduced into isolated stromal cells using a retrovirus vector and the transduced stromal cells have been used to effectively treat rats in an art-recognized rat model of Parkinson's disease.

In addition, the specification at pages 40-42, discloses various well-known methods for introducing an isolated nucleic acid into an isolated stromal cell (*e.g.*, electroporation, retrovirus infection, lipofectamine transfection, calcium phosphate, DEAE dextran, nuclear injection, and the like) as well as assays to determine the successful expression as well as the level of expression of the therapeutic protein encoded thereby (specification at pages 42-56). *See also* Schwarz et al., *supra*.

Therefore, given the disclosure provided in the specification and the state of knowledge in the art, claim 17 is in no way vague and indefinite with regard to which nucleic acid is to be introduced into the cells or how it is to be introduced, and this rejection under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

Claim 18 stands rejected as vague and indefinite under 35 U.S.C. §112, second paragraph, because, in the Examiner's opinion, it is unclear what is meant by the phrase "immunologically isolated" because "it is unclear if any 'type' of immunologically isolated cell is suitable for the treatment of any disorder, condition or disease of the central nervous system" (Office Action at page 8). The Examiner appears to be making a 35 U.S.C. §101 lack of utility rejection in that she asserts, in essence, that it is unclear whether the invention can work at all for any CNS disease, disorder or condition; however, the Examiner has framed this Section 101 rejection under the guise of a Section 112, second paragraph, indefiniteness rejection which is not allowed under the patent statute.

In any event, Applicants respectfully submit that claim 18 is not vague and indefinite for purposes of 35 U.S.C. §112, second paragraph. More specifically, "immunologically isolated" is defined in the specification at page 11, lines 17-28. The specification also makes clear that separating the implanted stromal cell from the recipient immune system protects the cell from elimination by the immune system. Methods for maintaining implanted stromal cells immunologically isolated from the host immune system are well-known in the art and are also disclosed in the specification as filed at pages 45-47. Indeed, diffusion chambers are commercially available (specification at page 45, lines 26-28) and their design is well-known in the art (specification at page 45, line 28, to page 47, line 2). Indeed, a typical diffusion chamber design is depicted in Figure 2 of the specification and is described at page 46. The specification makes clear that immunologically isolated cells can be used whenever separation of the cells from the recipient's immune system is desired. Based

upon the disclosure provided in the specification and the knowledge of those skilled in the art, one skilled in the art would understand which type of immunologically isolated cell is suitable for treatment of a CNS disease, disorder, or condition. Thus, there is no basis for rejecting claim 18 under 35 U.S.C. §112, second paragraph, since the claim is in no way vague and indefinite.

The Examiner contends that claim 19 is vague and indefinite since, in the Examiner's view, it unclear what is intended by "substantially homogeneous," it is unclear which differentiated cells are required or what the source of the cells is, and it is unclear how one determines whether the isolated stem cells acquire the phenotypic characteristics of the differentiated cell.

As stated previously elsewhere herein, the term "substantially homogeneous population of differentiated cells" is defined in the specification at page 14, lines 3-5. The term means a population of cells "wherein at least 75% of the cells exhibit the same differentiated phenotype" thus this is not a "relative" term as urged by the Examiner. Instead, this is an objective quantifiable determination which can be made by examining the cells. Thus, there is nothing vague or indefinite about this term.

Further, given the state of the art where cells can be readily selected and isolated based on a wide variety of phenotypic characteristics (*e.g.*, cell morphology, adherence to certain substrates, labeling by surface markers combined with cell sorting, negative or positive selection methods, and the like), one of ordinary skill in the art would not be confused by the term "substantially homogeneous" as defined in the specification.

Nor would the skilled artisan be confused as to which differentiated cells are required or how to obtain such a cell population from a source. That is, the specification makes clear that the differentiated cells direct differentiation (as that term is defined in the specification at page 14, lines 6-9) so that the isolated stem cells cocultured with the differentiated cells exhibit a phenotypic characteristic of the same

cell type as the differentiated cells. Indeed, as pointed out previously elsewhere herein, the invention has been extensively reduced to practice including coculturing isolated stromal cells with a substantially homogeneous population of astrocytes thereby mediating pre-differentiation of an isolated stromal cell into cell having at least one characteristic of an astrocyte (*e.g.*, expression by the cells of glial fibrillary acidic protein; GFAP) (*see* Example 8 at pages 53-56). The specification also teaches how primary cultures of astrocytes were obtained from a source (specification at page 48, lines 11-28).

Also, there is nothing unclear about which differentiated cells are required in the method. This is because the invention is directed to treatment of CNS diseases, disorders, or conditions such that the type of cell must be germane to transplantation in the CNS and therefore the types of cells required in the method is limited. One skilled in the art would not be confused as to which differentiated cells are required to effect directed differentiation of stem cells to a desired cell type using the methods disclosed in the present application which have been used to reduce the invention to practice. Therefore, in light of the extensive reduction to practice in this application and the state of the art, there is nothing vague or indefinite about which differentiated cells are required in the method or the source from which the cell can be obtained.

It is not unclear which phenotypic characteristics should be analyzed to determine whether the isolated stem cells acquire the phenotypic characteristics of the differentiated cells. Phenotypic characteristics, as the term is defined in the specification, includes "at least one of the following characteristics: morphological appearance, the expression of a specific protein, a staining pattern, and the ability to be stained with a substance" (specification at page 13, line 27, to page 14, line 2). Thus, the term is defined clearly in the specification. Moreover, the specification discloses numerous phenotypic characteristics which can be used to determine whether the isolated stem cell acquires the phenotypic characteristic of the differentiated cell (*e.g.*,

expression of glial fibrillary acidic protein, fibronectin, collagen I, HLA-ABC, vimentin, galactocerebrosidase C, and von Willenbrand Factor). Further, one skilled in the art would appreciate, based upon the disclosure provided in the specification, that the specific phenotypic characteristics will vary according to which differentiated cell type is cocultured with the isolated stem cells and which cell type the stromal cell is being directed to differentiate into. Several phenotypic characteristics and various assays to evaluate the presence thereof are disclosed in the specification at pages 50-56, or are well-known to one of ordinary skill in the art.

Similarly, with regard to the Examiner's opinion that claim 16 is indefinite because it is unclear what is required in the culture steps to direct differentiation, what is intended by "substantially homogeneous" and the method of obtaining the substantially homogeneous population, and which differentiated cells are required (as well as their source), all of these teachings are set forth in the specification as filed and/or are well-known in the art. Indeed, as stated previously elsewhere herein, the directed pre-differentiation of isolated stromal cells into cells comprising markers and phenotypic characteristics of astrocytes by coculturing the isolated stromal cells with a substantially homogeneous population of astrocytes has been reduced to practice.

Additionally, the term "substantially homogeneous population of differentiated cells" is defined in the specification at page 14, lines 3-5. The term means a population of cells "wherein at least 75% of the cells exhibit the same differentiated phenotype." Given the state of the art where cells can be readily selected and isolated based on characteristics (*e.g.*, cell sorting using surface markers, and the like), one of ordinary skill in the art would not be confused by the term "substantially homogeneous" as defined in the specification nor would the skilled artisan be confused as to how to obtain such a cell population from a source. Indeed, as pointed out previously elsewhere herein, the invention has been extensively reduced to practice

including coculturing isolated stromal cells with a substantially homogeneous population of astrocytes thereby mediating pre-differentiation of MSCs into cells having at least one phenotypic characteristic of an astrocyte (*i.e.*, expression of glial fribillary acidic protein).

Therefore, given the extensive reduction to practice in the instant application and the knowledge of the art, claim 19 is not vague and indefinite in any way and the rejection of this claim under 35 U.S.C. §112, second paragraph, should be reconsidered and withdrawn.

For the reasons set forth above, claims 1-20 are not vague and indefinite under 35 U.S.C. § 112, second paragraph, and the rejection of these claims on that basis should be reconsidered and withdrawn.

Rejection of Claims 19 and 20, Under 35 U.S.C. § 103(a)

Claims 19 and 20 stand rejected under 35 U.S.C. § 103(a) as apparently being, in the Examiner's view, unpatentable over Eglitis et al. (1997, Proc. Natl. Acad. Sci. USA 94:4080-4085), taken with Pereira et al. (1995, Proc. Natl. Acad. Sci. USA 92:4857-4861), Friedmann (1994, TIG 10:210-214), and Prockop (1997, Science 276:71-74). The Examiner contends that Eglitis discloses that transplantation of a population of vector-tagged bone marrow cells into a recipient results in subependymal concentration of marrow-derived cells. According to the Examiner, Eglitis indicates that the subependymal zone is an important source of neuronal and glial progenitors therefore finding bone-marrow derived cells there makes possible that the cells receive cues guiding their differentiation once they enter the brain. Further, the Examiner contends that Eglitis teaches that marrow-derived progenitors are not restricted to differentiate into a particular subclass of astroglia as demonstrated by the fact that glial fibrillary acidic protein (GFAP) staining of male donor cells was detected in both gray and white matter.

The Examiner then combines the teachings of Eglitis with those of Pereira and Friedman to somehow arrive at the conclusion that the combination of Eglitis with those references renders co-culture of isolated MSCs with differentiated cells, *e.g.*, astrocytes (claim 20), to direct their differentiation into a specific cell type, *e.g.*, astrocytes, *prima facie* obvious. Further, the Examiner contends that the afore-mentioned references combined with Prockop would cause one of ordinary skill the art to have a high expectation of success for establishing the *in vitro* conditions permissive for directing the differentiation of marrow-derived cells into specific cell lineages, such as astrocytes. This is so because, in the Examiner's opinion, Prockop teaches differentiation of marrow-derived cells into osteoblasts, chondrocytes, and adipocytes in culture such that directing differentiation of marrow-derived cells into astrocytes *in vitro* would be *prima facie* obvious.

Preliminarily, the three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

Eglitis, combined with Pereira, Friedmann, and Prockop does not teach or suggest all of the claim limitations. More specifically, claims 19 and 20 recite that the method of directing the differentiation of an isolated stromal cell comprises culturing the cell in the presence of a population of differentiated cells whereby the

stromal cell differentiates into a cell of the same type as the cells it is co-cultured with. The combination of references urged by the Examiner does not teach this claim limitation. Indeed, Eglitis, Pereira and Friedmann do not mention any methods of *in vitro* culturing isolated stromal cells much less methods of co-culturing the cells with other cells. Eglitis and Pereira disclose only the administration of bone marrow-derived cells from a donor and tracing their subsequent fate in a recipient animal (*e.g.* where do the cells localize and what, if any, cell lineage-specific markers do they express). Therefore, Eglitis and Pereria have nothing whatsoever to do with culturing isolated stromal cells or directing their differentiation in any way.

Similarly, Friedmann's discussion of stromal cells is limited to two paragraphs at page 212. In these paragraphs, Friedmann does not mention directing the differentiation of stromal cells at all but only discusses that since the mammalian CNS contains cells probably derived from bone marrow, bone marrow may be a useful source of cells which can be genetically engineered and introduced into the CNS to deliver a therapeutic gene product. There is no discussion in Friedmann concerning differentiation of stromal cells much less how this might be directed *in vitro*.

Prockop cannot correct the deficiencies of Eglitis, Pereira and Friedmann since, as pointed out previously elsewhere herein, Prockop is not a prior art reference for purposes of 35 U.S.C. §103(a) since it was published less than one year before the filing date of the instant application, *i.e.*, February 24, 1998. Therefore, whatever the deficiencies of combining Eglitis, Pereira, and Friedmann, these deficiencies cannot be corrected by combining them with Prockop.

Further, there would have been no motivation to combine Eglitis, Pereira, and Friedmann to produce a method of directing stromal cell differentiation *in vitro* by co-culturing the stromal cell with differentiated cells. This is because Eglitis, neither alone nor combined with other references, teaches or suggests directed differentiation of an isolated stromal cell and certainly does not teach or suggest how to direct differentiation *in vitro* by co-culturing with a substantially homogeneous

population of cells. Moreover, Pereira and Friedman also do not teach or suggest that stromal cell differentiation can be directed much less how such differentiation would be accomplished in culture. In addition, Prockop is not prior art for purposes of 35 U.S.C. §103. Therefore, there would be no motivation to combine these references since the combination does not teach or suggest that stromal cells can be caused to differentiate by co-culturing them with differentiated cells of a desired cell type.

In light of the foregoing arguments, it is clear that there was no reasonable expectation of success in combining the references to devise a method to direct differentiation of stromal cells by co-culturing them with a substantially homogenous population of differentiated cells of the desired cell type. That is, a person of ordinary skill in the art would not expect to succeed in directing differentiation of stromal cells by co-culturing the cells with a population of differentiated cells by combining references that have no suggestion or teaching as to how to direct differentiation of stromal cells *in vitro* using co-culturing them with other cells. As discussed previously elsewhere herein, Eglitis, Pereira and Friedmann do not discuss directing stromal cell differentiation at all; instead, these references note that bone marrow-derived cells implanted into a recipient animal can be found in certain tissues and express cell lineage-specific markers in those tissues. Nowhere in these references is there a teaching or suggestion that differentiation can be achieved by co-culturing stromal cells with a population of differentiated cells. Thus, there could be no reasonable expectation of success that combining Eglitis with Pereira, and Friedmann would result in the present invention.

For the reasons discussed above, the combination of Eglitis with Pereira, and Friedmann (Prockop cannot be combined since it is not prior art), cannot render claims 19 and 20 *prima facie* obvious under 35 U.S.C. § 103(a) and, therefore, the rejection should be reconsidered and withdrawn.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been either overcome or is now inapplicable, and that each of claims 1-20, is in condition for allowance. Reconsideration and allowance of each of these claims are respectfully requested at the earliest possible date.

Respectfully submitted,

DARWIN J. PROCKOP *ET AL.*

February 10, 2000
(Date)

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Enc. Copy of IDS filed May 15, 1998
Copy of Stamped Post Card showing receipt of IDS by PTO in May, 1998
Schwarz et al. (1999, Hum. Gene Ther. 10:2539-2549)
Two Month Petition for Extension of Time

KDL

CSK

ATTORNEY-DOCKET # 9598-32 TODAY'S MAILING DATE 5/15/98

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OF: Darwin J. Prockop et al.

FOR: ISOLATED STROMAL CELLS FOR USE IN THE TREAT-MENT OF DISEASES....

RECEIPT IS ACKNOWLEDGED FOR THE FOLLOWING:

TRADEMARK APPLICATION _____	COVER SHEET/LETTER/ORDER
_____ Specimens	AMENDMENT/REQ. RECONS
RENEWAL/AFF 8/AFF 8 & 15	ASSIGN/CHG NAME/MERGER/SEC. INT.
PAT APP (NEW/DIV/CIP/FWC/RE)	VER STMT/SMALL ENTITY
_____ Dec. & Power of Atty	NOTICE OF APPEAL/BRIEF _____ COPIES
_____ Pages Specifications	FINAL FEE
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_____ Claims	INF. DISCL. STATEMENT PTO Form 1449*
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Fee authorization charge Deposit Acct. #16-0235 $ _____

OTHER (PAPER TITLE) * and (1) copy of each ref. listed